6-1977

An Analysis of Species Recognition in Sympatric, Allopatric and Reciprocally Cross-fostered PEROMYSCUS CALIFORNICUS and PEROMYSCUS EREMICUS (Rodentia, Cricetidae)

Ronald L. Carter

Abstract

AN ANALYSIS OF SPECIES RECOGNITION IN SYMPATRIC, ALLOPATRIC AND RECIPROCALLY CROSS-FOSTERED PEROMYSCUS CALIFORNICUS AND PEROMYSCUS EREMICUS (RODENTIA, CRICETIDAE)

by Ronald L. Carter

Experiments were performed to compare homospecific and heterospecific mate selection in two closely related species, Peromyscus californicus and Peromyscus eremicus. Comparisons were made between allopatric and sympatric populations and between males and females for mate selection performance. Both species made significant choice for the homospecific chambers. Significant homospecific choice was made by mice from sympatric populations, whereas allopatric populations did not demonstrate significant choice. No significant difference in choice performance was demonstrated between males and females even when the estrus stages of the females were controlled.

A comparison of different testing lengths and temporal regimes of data collection was performed with the result being a recommendation for data collection during the first ninety minutes of the testing period, or during longer periods, with analysis based on an average

for the entire test. The dependent variable, "initial choice", was correlated with results throughout the experiment.

Reciprocal cross-fostering between the two species resulted in significant choice for the heterospecific cross-foster species type by P. eremicus, but the species choice exhibited by cross-fostered P. californicus was not significantly different from random. Lab-reared controls chose significantly for the homospecific chamber. Behavioral and ecological differences between species were discussed in an attempt to explain possible reasons for the differential species response to cross-fostering.

LOMA LINDA UNIVERSITY

Graduate School

AN ANALYSIS OF SPECIES RECOGNITION IN SYMPATRIC, ALLOPATRIC AND RECIPROCALLY CROSS-FOSTERED PEROMYSCUS CALIFORNICUS AND PEROMYSCUS EREMICUS (RODENTIA, CRICETIDAE)

by

Ronald L. Carter

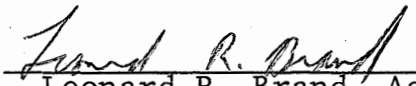
A Dissertation in Partial Fulfillment

of the Requirements for the degree

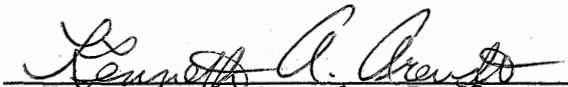
Doctor of Philosophy in the Field of Biology

June 1977

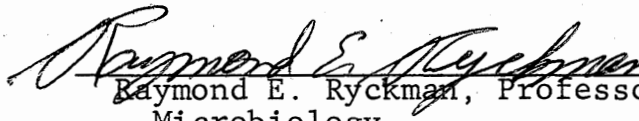
Each person whose signature appears below certifies that this dissertation in his opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.



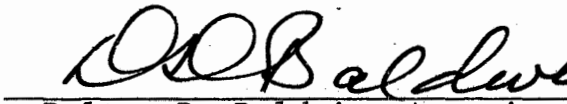
Leonard R. Brand, Associate Professor of Biology, Chairman



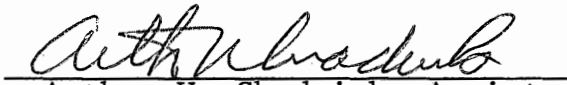
Kenneth A. Arendt, Professor of Physiology and Biophysics



Raymond E. Ryckman, Professor of Microbiology



Dalton D. Baldwin, Associate Professor of Christian Theology



Arthur V. Chadwick, Assistant Professor of Biology

Dedicated,

with all my love

to my patient and supporting wife, Kathy.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to the guidance committee members, Drs. Leonard R. Brand, Kenneth A. Arendt, Raymond E. Ryckman, Dalton D. Baldwin, and Arthur V. Chadwick, for their assistance and councel, and to all the students and staff in the Loma Linda University Biology Department who have in various ways contributed to my education.

Computational assistance was received from the Loma Linda University Scientific Computation Facility supported in part by NIH Grant RR-00276, and from Loma Linda University Medical Center Data Processing.

I am deeply grateful to Kathy Carter, Drs. Leonard R. Brand and Arthur V. Chadwick for their assistance in the preparation of this manuscript, and to Mr. and Mrs. W.A. Solomon for their educational assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The theory of organic evolution so central to the thinking of modern science needs to be studied and tested from many different disciplines and schools of thought. The process of "speciation" is the vehicle by which evolution proceeds. Fundamental to speciation is the establishment of reproductively isolated populations (Mayr, 1963). Non-interbreeding populations may then be molded differentially through selection pressures unique to each breeding group or deme. Oceans, mountain ranges, and glaciers are typical examples of extrinsic barriers to reproduction. Ethological barriers to reproduction are intrinsic isolating mechanisms that have been long observed, yet until recently few attempts have been made to quantify their importance to the process of speciation.

Early experiments demonstrating the ability of certain species of Peromyscus (white footed mouse) to discriminate between their own (homospecific) and related (heterospecific) populations were conducted by placing a male and a female of each of two species into a cage consisting of four compartments (Blair and Howard, 1944; and Blair, 1953). Social combinations involving the association of a male and female of the same species, based on daily records of the distribution of the four mice, occurred much more

frequently than would be expected under random distribution. Harris (1954) carried the research to the subspecific level by attempting to demonstrate the occurrence of assortive mating and sexual isolation between two subspecies of Peromyscus maniculatus. His testing procedure differed from Blair (1953) and Blair and Howard (1944) in that he used 3 compartments occupied by a total of three mice consisting of one individual from one of the two subspecies and one mouse of the opposite sex from each of the two subspecies. Harris demonstrated a tendency for males and females of the same subspecies to associate more often than males and females of different subspecies. However, no convincing statistics were presented. Tamsitt (1961), using a cage with four compartments, evaluated the presence or absence of species discrimination, male dominance, and gregariousness within the species and subspecies levels for Peromyscus nasutus, Peromyscus comanche, and Peromyscus truei. One conclusion was that P. truei does not discriminate within its own intraspecific population.

Moore (1965) tested allopatric populations of P. maniculatus and Peromyscus polionotus and suggested that the high species recognition performance of the former and low performance of the latter was due to the degree of geographic isolation of the two species. Moore's experiments were conducted in a three compartmented choice box in which

stimulus producing animals were kept in the two outer chambers for eight hours. The experimental animal was allowed to enter the end compartments only after the stimulus mice were removed. Visual and auditory cues were therefore removed. Smith (1965) extended the study of sexual isolation among natural populations by comparing allopatric and sympatric populations of Peromyscus californicus and Peromyscus eremicus males. No difference in the species recognition performance between allopatric and sympatric male P. californicus was found. There was however a difference between allopatric and sympatric performance in P. eremicus. Smith modified the three compartment choice cage by adding two end compartments that housed the stimulus females throughout the experimental period. Contact between the test male and stimulus females was prohibited by double wire mesh screening.

Doty (1972) using a two odor, forced choice preference situation demonstrated that the odor preference of female P. maniculatus bairdi between male mouse odors of P. m. bairdi or P. leucopus noveboracensis was a function of estrus state. These tests were conducted in the absence of male stimulus animals. Urine and nesting material collected prior to testing were used as stimuli.

Numerous studies have been conducted demonstrating mammalian odors as chemical communicators (Beach, 1942; and Beach and Gilmore, 1949). Carr and Caul (1962) using

olfactory discrimination apparatus tested normal male rats which were prepuberally castrated and trained to discriminate between the odors from receptive versus nonreceptive females. Normal and ovariectomized female rats were trained to discriminate between the odors from normal versus castrated males. Dagg and Windsor (1971) have demonstrated that rodents can detect by smell minute traces of certain substances like homospecific urine diluted to 100 ppm in water.

Another line of research reviewed by Parkes and Bruce (1961) deals with the role of olfactory stimuli in the neurohumoral mechanisms. Van der Lee and Boot (1955) suggested that odor produced pseudopregnancies in crowded groups of female mice. Parkes and Bruce (1961) demonstrated that odor of strange males can block pregnancies in mice. Sexual readiness is also communicated by odor (Carr and Caul, 1962). Bowers and Alexander (1967) have shown that mice can recognize individuals by olfaction. An animal's dominance is reported to be communicated by olfaction also (Todd, et al., 1967). Whitten and Bronson (1970) have demonstrated that odors synchronize the estrus cycles of female mice. McClintock (1971) has suggested that estrus synchrony in human females can also be altered by homospecific odors.

Research on the development of species recognition and preference in mammals has recently been directed toward

manipulation of the early environment (Marr and Gardner, 1965). A process which restricts social preference to a specific class of objects is generally referred to as "imprinting" (Bateson, 1966). Most of the early imprinting studies were performed on birds. Whitman (1919) pointed out that if a bird is hatched and reared by a foster species it will prefer to mate with that species when fully grown. The effects of this process have been known for many years (Spalding, 1873; and Heinroth, 1911) and have recently been demonstrated with gulls (Harris, 1970). It was, however, Lorenz (1935) emphasizing the importance of the imprinting role in mate selection and its fundamental uniqueness in the learning process who brought the subject widespread attention. There have been numerous definitions for the process. Moltz (1960) defines imprinting as a procedure which has been found to evoke close following activities to the object which has been imprinted upon. Scott (1962) and Sluckin (1965) have limited their definitions to topographical characteristics of following. These authors do make their definitions broader in that they indicate many attachments are revealed by responses other than following.

Considerable controversy concerning the definition and process of imprinting has occurred since Lorenz put forth his imprinting theory (1937). Moltz (1960), Sluckin and Salazen (1961), Fabricius (1962), and Hinde (1962) in

particular have questioned Lorenz's theory. Due to current controversies the term "imprinting" is still ill-defined.

Lorenz (1937), Tinbergen (1951, and 1953), Freud (1949), and Fenichel (1945) provide several theoretical positions on the imprinting phenomenon that are appropriate to this study. Moltz (1960) and Sluckin (1965) provide excellent reviews of the imprinting literature.

Marr and Gardner (1965) have shown that specific social-behavioral patterns are a function of early olfactory experience in the rat. Mothers and young were rubbed daily with cloths smelling of normal odor, cologne or methyl salicylate. After the young were reared in this regime the cologne group preferred cologne rats. The sexual responsiveness of subjects reared with mothers of other than normal odor was significantly less than the sexual responsiveness of the subjects reared with mothers of normal odor.

Attempts have been made to modify the growth and behavior of rat pups by the experimental manipulation of the mother (Denenberg et al., 1962; and 1963). Through different forms of conditioning Denenberg has demonstrated that experiences which the mother received while an infant were profound enough to modify her offsprings' body weight at weaning, and open field behavior in adulthood.

The species, number, and sex of littermates have also

been shown to affect adult behavior of certain rodents (Brain and Griffin, 1970; and Grota, 1973). Mice reared with rats showed significantly altered behavioral patterns, including the behavior of fighting, which is presumed to be adaptive to the mouse (Denenberg et al., 1964).

Cross-fostering experiments have provided additional information on learning and mate preference. The cross-fostering procedure has been useful as a control for the confounding problems produced by variations in parental environment which are correlated with genotype (Ressler, 1962).

Cross-fostering has also been useful in controlling for nutritional factors correlated with the behavior of the mother (Rosenberg et al., 1970). Rattus reared Mus have been found to be less aggressive in adulthood, to be less active in the open field, to have a lesser adrenocortical response to a novel stimulus, and most importantly to prefer a rat to a mouse in a two-choice social preference test (Denenberg et al., 1964, and 1968; and Hudgens et al., 1967, and 1968). Such research has provided further support for the hypothesis that the differences observed between mice reared with rats and control mice are behaviorally mediated and strongly suggests that the magnitude of these differences may be a direct function of the amount of contact between the rat foster parent and the mouse pup.

Literature concerning cross-fostering in rodents has

been almost totally restricted to laboratory mice and rats. Quadagno and Banks (1970) provide one of the few exceptions. Their work dealt with the effect of reciprocal cross-fostering between Baiomys and Mus musculus. The cross-fostered males and female Baiomys differed in that the females spent significantly less time adjacent to the conspecific than did the controls, whereas the males did not differ significantly from their controls thus indicating that females were more affected by cross-fostering than males. Walter (1973) found the opposite response in Zebra finch. Zebra finch males were shown to have learned species recognition in their nests while females were not affected by postnatal learning.

The purpose of this study is to further our knowledge about the involvement of learning in species recognition and ultimately in the process of speciation by first re-investigating mate selection in P. californicus and P. eremicus (Smith, 1965), adding certain important environmental controls and considering the estrus condition of the female mice. Secondly, this study will attempt to quantify the species recognition performance of females as well as males from both species. A third item of study will be to make comparisons of testing lengths to help answer some of the conflicting views about the length of testing needed to establish species recognition experimentally. A final purpose of this study

will be to produce reciprocally cross-fostered mice and to assess any postnatal effects on the mate preference of the cross-fostered mice.

MATERIALS AND METHODS

Experimental Subjects

Experiments were conducted using two species, Peromyscus californicus and Peromyscus eremicus of the subgenus Haplomylomys.

These experimental animals were chosen because Peromyscus are the most widely studied wild rodent (King, 1968), and because Peromyscus are easily maintained in the laboratory and will reproduce in captivity (Brand and Ryckman, 1969). Peromyscus californicus and P. eremicus are closely related (Hall and Kelson, 1959; and Osgood, 1909) and share a number of distinct subgeneric developmental and behavioral characteristics (King, 1968). Trapping records of Osgood (1909) and distribution maps (Hall and Kelson, 1959; and Ingles, 1965) show these species living sympatrically and allopatrically in areas close to Loma Linda University, Loma Linda, California (Fig. 1), where the experiments were conducted.

Peromyscus californicus are found in the chaparral (upper and lower Sonoran zones) of the western valleys and foothill woodlands of California and southward into lower California (Osgood, 1909). Osgood reports P. eremicus as being found in southern California from the western side of the southern California mountains

to Los Angeles and south through southwestern California. Peromyscus eremicus are most often associated with rocky out-croppings and build simply constructed nests. Peromyscus californicus construct elaborate nests from gathered woody vegetation or often inhabit abandoned woodrat (Neotoma) lodges.

These species are easily identified by numerous body measurements and coloring (Ingles, 1965; see appendix A for detailed descriptions for both species).

All experimental subjects were caught in aluminum Sherman live traps between December 1970 and June 1975, in the following California locations:

In Riverside County:

1. Three miles north of Sunnymead.
2. Four miles east of Sunnymead.

In San Bernardino County:

1. Twelve miles southwest of Hesperia or one east, 3/4 miles north of Cajon Pass Junction.
2. Three miles south, one mile east of Yucaipa.
3. Four miles south of Hemet.
4. Four miles north, 1.5 miles east of Highland.

Location number four in San Bernardino County provided the major source of P. californicus and P. eremicus in a sympatric situation. Peromyscus eremicus were found allopatrically in both Riverside County locations while sites two and three in San Bernardino County provided

allopatric P. californicus. All experimental subjects were wild caught except for the lab-reared controls and cross-fostered individuals.

Trapped mice were taken directly to the animal rooms in the Biology department of Loma Linda University where they were identified and placed one per cage into plastic planter boxes (13.5 cm x 14 cm x 40.5 cm) covered by 0.64 cm wire mesh tops. Animals were given at least one week to acclimate to the surroundings before they were used in an experiment. Each cage was provided with food and water ad libidum. Feed consisted of Purina Rat Chow and a mixture of rolled oats, cracked corn and bird seed. Fresh lettuce and dog food were periodically provided. Fresh pine shavings and paper towels were regularly provided for bedding. The animal rooms were maintained at 23° C, with the lights turned on from 0600-1800 Pacific daylight time.

Apparatus

Experiments were performed in six testing units, each consisting of five linearly arranged chambers, made of plexiglas with 0.64 cm wire mesh tops. Design of the units was similar to that used by Smith (1965). The two outer compartments for each unit housed the stimulus-providing mice (heterospecific and homospecific mice of the opposite sex from the test mouse). The end compartments were

separated from the center three chambers by two 0.64 cm wire mesh barriers. The center three chambers were connected by two tunnels which housed treadles (Fig. 2). When a treadle was moved allowing passage of the test mouse to the adjacent chamber, this event was recorded (via magnetic reed switches) in two ways. An Angus Esterline multi-channel event recorder provided continuous recording of all treadle flips and time spent in each chamber throughout the entire experimental period. An electric clock on another circuit recorded total time spent in each chamber. Data from the clocks for each chamber were recorded on film each hour by a motor driven, clock controlled 35mm camera (Fig. 2).

Experiments were conducted in environmentally controlled animal behavior rooms with a constant temperature of 23° C, and a light dark cycle with a light period of 0600-1800 PDT. During the day four 75 watt ceiling lights provided a light range of 100-700 lux. A single 75 watt bulb housed in a light diffusing shade provided an artifical moon of 0.1 to 1 lux. Temperature control fans provided a constant "white noise".

Procedure

Experiments were begun at 1800 PDT by placing a previously untested male or female mouse into the center chamber of the experimental unit. Treadle entries to the adjacent chambers were blocked by sliding barriers for a

period of twenty-four hours. During the acclimation period and throughout the entire testing time the center chamber provided food and water, with water available only in the center chamber. This acclimation period in the center chamber allowed time for the experimental organism to become familiar with the novel environment, and provided through exposure a preference for the center chamber, in the event that the first chamber encountered should influence preference. Choice for adjacent chambers would then require a distinct preference over the center chamber. At 1800 PDT following the acclimation period stimulus mice were placed in the detachable end compartments, and the end compartments were placed in series with the center three chambers. Treadle barriers were then removed through slots in the walls of the center chamber and behavior recording devices were activated.

Daily records of the placement and construction of nest sites were made visually (during 71 tests) and vaginal smears were taken daily from the female mice in each chamber (see appendix B for vaginal smear procedure). At the end of each experiment the animals were returned to the animal care rooms and the experimental units were thoroughly washed in hot detergent water with a brush. The units were rinsed in hot water and sprayed with a high pressure steam hose and allowed to air dry.

Treatment I (wild caught mice) consisted of 131 tests. Treatment II (cross-fostered and lab-reared controls) consisted of 44 tests. In all 175 experiments, mice were only tested once.

Data were analyzed statistically with chi square, paired-t, analysis of variance, and multivariant analysis through the use of the General Linear Hypothesis equation.

Cross-fostering procedure

Breeding pairs of wild caught mice were established in the winter of 1974. Daily observations were made to discover and record births. When concurrent (within 24 hrs.) births took place in both species, reciprocal cross-fostering was attempted. Births occurring without a heterospecific counterpart were used as lab-reared controls.

At the time of attempted cross-fostering, adult males were permanently removed. Pups from both species were removed from their mothers and given to heterospecific foster mothers within 36 hours after their birth. Pups were handled gently with sterile surgical gloves to eliminate human odor from being transferred with the pups. Prior to pup transfer sterile cotton swabs were used to collect odor (urine and vaginal discharge matter when available) from the foster mother. Odor laden swabs were rubbed over the bodies of the foster pups. An additional procedure used for approximately the last half of the

experiments that seemed to help make the mothers more receptive to cross-fostering was to remove the mothers from their cages when removing the genetic pups. Pups were gently removed from their mothers. Great care was used in this procedure since the pups hold on to their mothers teats with their milk teeth. Foster mothers were then simultaneously returned to their nests with the foster pups.

Foster pups as well as the laboratory reared controls were removed from their mothers of foster mothers upon completion of weaning. Siblings were caged together until sexual maturity and were then caged individually. Control and cross-fostered animals were given choice preference tests identical in procedure to the wild caught mice. Tests were conducted after the mice were determined to be sexually mature by the presence of regular estrus cycling for females and for males by age and testicle size.

RESULTS

Treatment I. (Wild Caught Peromyscus)

When all of the data from treatment I (n=131) were pooled, they revealed a significant overall choice for the chamber next to the homospecific animal (paired-t tests; $T=4.999$; $P<.0001$) (Fig. 3). This analysis was based on the number of minutes spent in each choice chamber.

Comparisons of sympatric and allopatric populations were made for the males of both species. Inadequate numbers of females from allopatric trapping sites made comparison of females impossible. When viewed separately ($P= .54$ and $P= .14$) or collectively ($P= .17$) allopatric P. californicus and P. eremicus showed (Fig. 4) a preference for the homospecific chamber which was not statistically significant at the P .05 level. However, sympatric females from both species made highly significant choices for the homospecific males ($P= .01$ and $P= .0001$) and sympatric males also chose homospecific females, but at a lower significance level ($P= .04$ and $P= .06$) (Fig. 4, table 1).

Figure 5 compares the amount of time spent in the choice chamber by different subgroups of the animals in treatment I (wild caught mice). There was no significant difference between the allopatric and sympatric mice in the

amount of time spent in either the homospecific (P= .06) or the heterospecific (P= .10) chamber. Similar comparisons between males and females also indicate no significant differences in amount of time in the homospecific (P= .98) or the heterospecific (P= .10) chamber. There is however, a significant difference between P. californicus and P. eremicus in the amount of time spent in the homospecific chamber (P= .02).

Table 2 compares the level of significance from paired-t tests of the choice for the homospecific chamber in four time periods: total length of experiment (60 hours); day time; night time; and first ninety minutes of testing. The level of choice was significant in nearly all of the subgroups (in the first four rows). The last two rows in table 2 compare day time choice to night time choice. This comparison was made in two ways; (1) comparison of percent homospecific choice in minutes, and (2) comparison of values derived by subtracting the time in the heterospecific chamber from the time in the homospecific chamber for each population. The second method was used in order to examine any possible effects due to differences in overall numbers of minutes involved in choice from animal to animal that might be masked by the comparison of a percent value. Neither of the two methods revealed any significant difference in homospecific choice between day and night.

Species preference was also analyzed for individual consecutive six-hour periods to determine if there was any pattern of change in choice performance with time. Species choice during the first ninety minutes and ten six-hour periods for the sympatric populations are plotted in figure 6. Choice during the first ninety minutes (which was part of the first six-hour time period) was highly significant for all subgroups. The total for the first six hours however was not significantly different from random.

Table 3 presents forty six-hour time periods (ten six-hour periods per subgroup). In all forty time periods except two, the mice in each population spent more than 50% of their choice time in the homospecific chamber. Peromyscus eremicus provided the two exceptions: during the first six-hour time period for males and during the fourth six-hour period for females. Unique to this first six-hour time period is the fact that not one of the four subgroups realized significant choice during this time block. Seventeen of the forty six-hour time periods showed significant homospecific choice ($P < .05$). Eleven of the seventeen significant periods were during the night hours (1800-0600 PDT). Eleven of the periods were significant for P. californicus homospecific choice. Data from table 3 would tend to suggest that choice improves from night to night. However, this is not readily seen in figure 6.

Data from table 2 suggest that there is no significant difference between choice made during the day and choice made during the night. Multivariant analysis through the use of the General Linear Hypothesis equation was performed on the mean homospecific choice, in minutes, for the total of the entire test period. This test also indicated significant overall choice for the homospecific animal ($F=4.5$; $P < .05$). Differences between species were also significant at the $P < .05$ level (table 4). The other variables of location (where the mouse was caught, with 6 separate locations); season in which the experiment was conducted; the "initial choice" and a species and "initial choice" interaction of variables were not significant.

However, when the data from only the first ninety minutes of each experiment were analyzed by the General Linear Hypothesis equation, the results were a little different from the results obtained from the 60-hour data. There was no difference between species, but the mean time in the homospecific chamber became even more highly significant, indicating distinct homospecific preference during the first 90 minutes for the pooled data. Location, season, and initial choice also were significant factors during the first ninety minutes but were not for the sixty hour data.

When individual six-hour time blocks were analyzed separately by multivariant analysis (General Linear

Hypothesis equation) (table 5) season, which had been shown to be a significant variable during the first ninety minutes was not seen as significant for any of the subsequent six-hour time blocks. Location which was also a significant variable for the first ninety minutes continued to be significant during the first six-hours. During a number of six-hour time periods the species variable was shown to be significant.

The initial choice made by a subject was recorded regardless of the amount of time spent subsequently in that choice chamber. The number of initial choices made for the homospecific and for the heterospecific chambers was analyzed by a non-parametric test (chi square distribution). The results ($P > .05$) suggest that the number of subjects making their first choice for the chamber next to the homospecific or next to the heterospecific animal was not significantly different from random. However, table 5 indicates that in most of the time blocks, initial choice was significantly related to subsequent choice levels.

Estrus state was analyzed during 71 experiments. Two way analysis of variance tests and multivariant analysis performed by the General Linear Hypothesis equation found estrus stages to be non-significant as a variable in overall species recognition. However, the estrus data did provide control for a large amount of variance within the analysis and greatly reduced the initial choice F value

from F = 23.88 to F = 8.07.

Treatment II. (Cross-fostered and laboratory reared controls)

The pooled data for cross-fostered mice were analyzed by paired-t comparisons for time in the homospecific chamber versus time in the heterospecific chamber (Fig. 7). The cross-fostered mice were the only population in both treatment I and treatment II that spent more time in the heterospecific than the homospecific chamber. However, this choice was not significant ($P > .20$).

The sympatric mice in treatment I spent significantly ($P = .00013$) more time ($x = 1203$ minutes) than the cross-fostered mice ($x = 593$ minutes) in the homospecific chamber. The number of minutes spent in choice by the cross-fostered mice was reduced. This seemed to be a characteristic difference between treatment I and treatment II. For example the sympatric mice of treatment I averaged 2,000 minutes in the choice chambers, while treatment II mice averaged 1,357 minutes in the choice chambers.

The choices of the two species of cross-fostered mice were analyzed separately and (because of the results in treatment I on testing length) only for the first ninety minutes (Fig. 8). Peromyscus californicus fostered by P. eremicus mothers showed random choice behavior with their mean average time spent in the chamber adjacent to the homospecific (genetic type). Peroymscus eremicus,

however, spent a significant (P= .03) amount of time next to the heterospecific mice (foster parent type). This was the opposite choice direction from the trend of the cross-fostered P. californicus.

Laboratory reared controls revealed significant choice for the homospecific chamber (P = .00019). A comparison of homospecific choice between controls and cross-fostered mice showed a significant difference in species recognition performance (P= .00008).

DISCUSSION AND CONCLUSION

Our results support Smith's general conclusions that P. californicus and P. eremicus demonstrate a preference for their own species, with mice from sympatric populations showing a more significant preference than mice from allopatric populations.

In the present study males of all subgroups spent more than fifty percent (Fig. 4; N=131) of the time in the homospecific chamber. This preference for the homospecific female was statistically significant for sympatric males of P. californicus and P. eremicus, but was not significant for allopatric males of either species. The amount of time spent next to the homospecific female, by allopatric males, showed great variability with standard errors up to three times the standard errors for sympatric males.

Smith (1965) using mice from a different locality, also found that P. eremicus and P. californicus from sympatric populations made a significant choice for a homospecific mate, but allopatric P. eremicus did not make a significant choice. Peromyscus californicus from allopatric populations made a significant choice for the homospecific mate in Smith's study, but not in the present study.

An even larger difference was evident between the

sympatric and allopatric populations in the amount of time spent next to the heterospecific female (allopatric, $\bar{x}$=985 minutes; sympatric, $\bar{x}$=622). Further study of this phenomenon may provide an insight into the basic behavioral differences between allopatric and sympatric populations.

Perhaps the differences between allopatric and sympatric responses to mate selection can be explained by a theory of speciation that would predict a selective advantage for sympatric populations having produced an efficient isolating mechanism that would maintain species integrity (Dobzhansky, 1940 and 1951). Differences between our results and those of Smith (1965) may be due to dissimilar selective pressures that are acting on isolating barriers within the two separate populations.

In our experiments, nest construction took place periodically and often nests were removed from chamber to chamber during the duration of the experiment. Smith (1965) found the location of the nest to be unchanging once contruction had occurred. This difference could be due to differences in experimental design. One major difference involves the time periods used for measurement. Smith recorded data on the third, fourth, and fifth experimental nights from 2200 to 0600 PDT. Our testing regime continuously measured choice for the entire sixty hours of testing with data collection beginning at 0600 PDT on the first day. A second difference in experimental design deals with the

manner in which the experiments began. Our mice were acclimated to the center chamber for twenty four hours prior to the commencement of mate preference tests. Only at the end of the acclimation period were the caged stimulus producing mice placed in linear arrangement with the three-chambered choice apparatus. Water was provided only in the center chamber, while food was provided in all three chambers in equal amounts. Smith began his experiments by randomly placing the experimental mouse in one of the three experimental chambers. The end compartments housing the females were already in series with the center chambers. Data collection was begun only after the experimental male mouse had been free to explore all three chambers for two days. Both food and water were available in all chambers. It would seem that a built-in preference for the center chamber (provided in our design) with its singular water supply acting as a reinforcement to the center chamber would require a stronger demonstration of heterospecific or homospecific choice.

Our data indicates that there is no significant difference between males and females in mate selection performance (table 4, and Fig. 5). This was an unexpected result, since it has been suggested that in non-monogamous animals, the female should have higher discrimination ability. It has been postulated for some time (Sibley, 1957; Selander, 1965) that in the evolution of secondary

sexual characteristics in non-monogamous species or species with a short "pairbond" there will be a greater premium upon rapid and correct species recognition. This reasoning has been especially applied to species in which males do not take an active part in nest building and care of the pups. In these cases of polygamous and promiscuous mating systems it is postulated that selection for species discrimination will act primarily on the female. It is further theorized that natural selection will only tend to suppress crossbreeding if those individuals which hybridize will in consequence pass on fewer gametes in the form of non-hybrid offspring. It is therefore believed that this would be more often the case in females than in males (Knight et al., 1956). Mayr (1963) states that "the male is almost invariably more active in courtship and, in virtually every case, less discriminating". Doty's work (1973) with Peromyscus and their reaction to homospecific and heterospecific urine odors supports the idea that there is a difference in mate selection performance between males and females at least in P. maniculatus. However, he was not able to demonstrate a similar difference in P. leucopus.

Because our results have shown no difference between males and females in discrimination ability, they may suggest that Peromyscus has a more highly structured social system than heretofore believed (Eisenberg, 1962 and 1963).

Our study quantified estrus states for both female experimental subjects and for stimulus providing females. The stage of estrus was found to be a non-significant variable (multivariant analysis) in either its effect on male preference or on female preference. Doty (1972) demonstrated that female P. maniculatus bairdi reacted to male P. m. bairdi and to P. leucopus noveboracensis as a function of gonadal state. However, our results support the conclusions of Carr and Caul (1962) who state that both the rate at which olfactory discrimination was established and the accuracy of the discrimination were independent of the gonadal state of both the male and female. Moore (1965) and Godfrey (1958) state similar conclusions for some species and the opposite conclusions for other species.

Differences between our results and those obtained by Doty (1972) may be due in part to differences in experimental design. Doty's work utilized two types of test apparatus, a choice box olfactorium and a "Y" type choice maze. In both cases the cue presented to the experimental animal was that of odor alone. Our experiments involved multi-modal stimuli from living animals who were separated only by wire screens. Therefore tactile cues were the only cues eliminated in our study. It would seem to this author that there might also be a selective advantage for species recognition when mating is not the

immediate response. It might well be that species recognition would be advantageous to any social structure. It is therefore conceivable that our results and those of Doty's are not contradictory. Taken together they may suggest a discrimination ability both for sexual readiness and for species recognition. This only serves to further emphasize the need for a careful re-evaluation of the term "mate preference" which has been loosely used to mean any behavior involved in reproduction, association of sexually unlike pairs (Blair and Howard, 1944), and time spent adjacent to caged animals (Smith, 1965; and this study) or time spent adjacent to some odor (Doty, 1972). The literature has not thus far clearly defined mate preference. Experiments should be conducted to critically evaluate distinctions between mate preference, species recognition, and inter as well as intra-species avoidance. This is not to say that previous experiments have not contributed to the topic of ethological mechanisms in reproductive isolation, since all of the concepts mentioned are probably involved to some degree.

Our data were analyzed in a number of different ways in an attempt to determine the most effective testing periods for mate selection studies. Choice expressed in mean number of minutes for the homospecific chamber was analyzed separately for days (0600-1800 PDT), nights (1800-0600 PDT), the first ninety minutes of testing, the

total sixty hour testing period, and individual six-hour blocks. Our data indicate that the following four data collecting regimes gave consistent results: (1) the first ninety minutes, (2) the total 60 hour test, (3) night data (three nights), (4) day data (2 days). The first ninety minute period as well as the sixty hour totals indicated significant homospecific choice. Both day and night data indicated significant choice, and did not significantly differ from each other.

No one day or night period showed consistent homospecific choice, and individual six-hour blocks did not give consistent results. Conclusions based on data accumulated for each consecutive six hours of the testing period suggest that choice does not improve as the experiment proceeds. The first six hours of testing appears to be the only unique six-hour period. This time period statistically reflected random activity for all subgroups ($P > .05$; paired-t test, and analysis of variance).

Multivariant analysis performed on the same data, for the first six hours by the General Linear Hypothesis equation indicated significant choice for the homospecific chamber. Location (sympatric or allopatric) and initial choice were shown to be significant variables affecting the results for this first six hours. Initial choice and species were both significant variables in most time periods. According to the General Linear Hypothesis

analysis the level of choice exhibited by sympatrics and allopatrics was significantly different during the first ninety minutes and throughout the remainder of the first six hours. Experiments conducted in different seasons of the year gave significantly different results with the difference being observable for the first ninety minutes and from the accumulated sixty hour data.

It is my conclusion based on the experiments investigating testing duration, that the first ninety minutes of experimentation provide reliable data that reflect accurately (in our tests) choice behavior that is observed during the subsequent sixty hour testing period. In addition to reliablity, significant differences in some design variables were demonstrated during the first ninety minutes, which are behaviorally interesting and are not observable during other time periods in our data.

Initial choice, the first chamber entered at the beginning of an experiment, quite consistently emerged from our results as a significant factor in data analysis. Non-parametric statistics ($P > .05$; Chi square distribution) indicated that the initial choice was not significantly different from random, in both pooled and subgrouped data and was not shown to be correlated to estrus states of the female. However, initial choice was related to subsequent choice. Those mice that made their initial choice for the homospecific chamber showed more significant subsequent

choice for the homospecific, whereas those that initially chose the heterospecific chamber showed less significant choice for the homospecific. Sex, species and location were equally represented in the two initial choice categories, and thus apparently are not the basis for the two groups.

The data seem to suggest that there are at least two observable groups of animals: those that demonstrate initial choice for homospecific and those that demonstrate initial choice for heterospecific animals. These two groups appear to respond to mate choice in a significantly different way throughout the experiment. Further study investigating possible behavior or genetic differences between these two groups may be very rewarding. The lack of correlation between initial choice and estrus stages would tend to suggest explanations for these groupings on behaviors other than sexual readiness. However, it must be noted that estrus states may not be a sensitive enough criterion for measuring sexual readiness. Our data suggest the possible existence of "high" and "low" mate discriminators within each population.

This study has successfully produced reciprocally cross-fostered P. californicus and P. eremicus which have been reared to sexual maturity. It is concluded that cross-fostering can significantly alter mate selection in at least one species of Peromyscus. These findings support

in general the cross-fostering studies on birds and extend our knowledge of the effects of cross-fostering to wild rodents.

Pooled data from cross-fostered P. eremicus and P. californicus indicated random choice with the mean choice in the first ninety minutes to be for the heterospecific. When the two species were analyzed separately, only one species was shown to have made significant choices. Peromyscus californicus spent more time in the chamber adjacent to the homospecific female, but this was not significant (P= .23). Conversely, P. eremicus showed significant preference for the heterospecific chamber (P< .02). It would appear that reciprocal cross-fostering has altered significantly the species preference of P. eremicus, and that preference for the foster parent type has persisted into sexual maturity. Peromyscus californicus were either not significantly affected in their mate selection behavior by cross-fostering or had lost the effects by the onset of sexual maturity.

Smith (1965) concluded that increased discrimination shown by sympatric P. eremicus as opposed to allopatric P. eremicus is probably not due to any learning mechanism (i.e., association or imprinting). He cites a number of authors (Blair and Howard, 1944; Blair, 1953; Harris, 1954; and Moore, 1965) whose studies tend to negate the idea

of learning by association. Animals which were removed at least one generation from the wild and with no previous experience with the other mice could select for the homospecific. Smith challenges the idea of imprinting, a special case of learning, as a feasible explanation for the discriminatory differences between sympatric and allopatric populations since this hypothesis would require some enviornmental factor which makes imprinting more effective in one locality. Such a factor he feels is doubtful.

Our experiments with P. eremicus have shown that the mate selection behavior at least within this population has a component that is modifiable by learning. On the other hand response of cross-fostered P. californicus was not altered to a significant degree suggesting genetic control for mate selection in this species.

There are a few ecological and behavioral differences between P. eremicus and P. californicus that may provide some understanding of the differential species response to reciprocal cross-fostering. First of all, the two species occur sympatrically in areas of dense chaparral. In the area of overlap, P. eremicus is found allopatrically where the cover is sparse, while P. californicus may be found allopatrically in some canyons where trees or large shrubs are the dominant type of vegetation. Eisenberg (1962, and 1963) defines a loose type of social structure for

P. eremicus and a higher social structure for P. californicus, determined on the basis of how they have adapted to different habitats. McCabe and Blanchard (1950) have stated that P. californicus females in comparison to female P. eremicus are more tolerant of young from previous litters and of the male during and after birth of new litters. Compared to other species in the genus, P. californicus build and defend nests for the longest periods of time. McCabe and Blanchard (1950) suggest that this is due to the fact that P. californicus have lower reproductive rates and are therefore more protective of their young. Consistent with this statement of behavior, McCabe and Blanchard have also described P. californicus as more aggressive than P. eremicus. Response of the foster mothers toward presentation of foster pups during our experiments are consistent with behavior patterns just described. The less aggressive P. eremicus with a lower level of social structure was much more accepting of heterospecific pups. Peromyscus californicus, the most aggressive defender of its nest and possessor of a higher social structure, was much less accepting of heterospecific foster pups. Possibly the same discriminating mechanism in P. californicus that allows acceptance of young from previous litters and males during and after birth of new litters, acts by discriminating against heterospecifics in order to maintain species integrity.

It is of great interest to observe a learning component involved in the phenomenon we call "mate preference" or "species recognition". Further study investigating the possible differential response to mate selection caused by cross-fostering within two closely related species may greatly add to our knowledge about the degree of and possible mechanisms involved in ethological isolation.

One might predict that there would be a selection advantage for a learning component in mate preference and/or species recognition for species that are geographically and behaviorally more diverse and less specialized. On the other hand one could expect an opposite selective advantage for species that are more specialized geographically and which are more defensive territorially as in the case of P. californicus.

A most important area of research purposed by this study is to investigate naturally occurring environmental pressures that may affect postnatal learning, especially learning that is involved in mate preference.

TABLE 1. A Comparison of Homospecific and Heterospecific Choice by Sympatric Mice Grouped According to Species and Sex. Data from entire 60 hour test period.

		P. californicus				*P. eremicus*			
		Females		Males		Females		Males	
		Homo-specific	Hetero-specific	Homo-specific	Hetero-specific	Homo-specific	Hetero-specific	Homo-specific	Hetero-specific
$\bar{X}$ Min. in Choice Chambers	$\bar{X}$ =	1354.8	659.1	1488.5	579.7	1021.0	615.0	941.1	605.5
	Se.=	356.0	80.5	253.3	399.5	157.6	119.0	235.0	118.6
	N =	27		14		22		16	
	P =	.0009		.04		.007		.06	

TABLE 2. Comparison of the Amount of Time Spent in Homospecific Chamber and the Amount Spent in Heterospecific Chamber for Pooled Data and Several Subgroups Presented as T-Values from Paired T-Tests

Minutes in homospecific vs. minutes in heterospecific chamber	Pooled data	Males	Females	P.californicus (♂,♀)	P.eremicus (♂,♀)
Total	4.999 ****	4.756 ****	2.838 **	4.675 ****	2.495 *
First 90 minutes	3.578 ***	3.276 **	1.958 +	2.732 **	1.677 +
Total day	4.205 ****	3.895 ***	2.504 *	3.591 ***	2.248 *
Total night	4.984 ****	4.102 ***	2.983 **	5.086 ****	1.806 +
Difference between night choice and day choice					
Minutes	1.463	1.508	0.473	2.352 +	0.073
Percent	0.415	0.105	0.505	0.156	0.504

P .1 = +
P .05= *
P .01= **
P .001 = ***
P .0001= ****

TABLE 3. Percent of Time in Consecutive 6 Hour Periods Spent in the Homospecific (first value in each block) and Heterospecific Chambers from Analysis of Variance.

		1st 90 min	1	2	3	4	5	6	7	8	9	10
californicus ♀	$\bar{X}$	62 38	57 43	71 29	68 32	64 36	57 43	67 33	67 33	65 35	64 36	56 44
	SE	4 4	5 5	5 4	6 6	7 7	7 6	6 6	6 6	7 7	6 6	7 7
		**		***	**	+		**	*	+	**	*
eremicus ♀	$\bar{X}$	64 36	54 46	61 39	61 39	45 55	56 33	63 37	51 49	73 27	56 44	66 34
	SE	5 5	8 8	9 9	9 9	8 8	7 7	6 6	8 8	9 8	9 8	8 7
		*		+			+	*		**		*
californicus ♂	$\bar{X}$	73 26	53 47	64 36	72 28	69 31	66 34	70 30	65 35	64 36	67 33	78 22
	SE	7 7	8 8	8 8	9 9	12 12	8 8	7 7	9 9	11 11	7 7	4 4
		*		+	*		*	**			*	****
eremicus ♂	$\bar{X}$	56 44	42 59	54 46	60 40	73 27	60 40	59 41	58 42	70 30	68 32	64 31
	SE	8 8	7 7	6 6	10 10	10 10	6 6	8 8	8 8	9 9	6 6	8 8
						*	+			*	**	

P .1 = +
P .05= *
P .01= **
P. .001 = ***
P. .0001= ****

TABLE 4 Multivariant Analysis (General Linear Hypothesis) Listing F-Value and Levels of Significance for Homospecific Choice, Independent Variables, "Initial Choice", and the Interaction of Initial Choice per Species for the First 90 Minutes and 60 Hour Total.

Variables	1st 90 min. F-Value	Total 60 hr. F-Value
Homospecific Choice	19.26****	4.50*
Species	0.86	3.72*
Locations (6)	2.45*	0.98
Season	4.65*	2.46*
Sex	0.66	0.10
Initial Choice	6.20**	2.98*
Species Initial Choice Interaction	2.0	0.12
	N = 131	N = 131

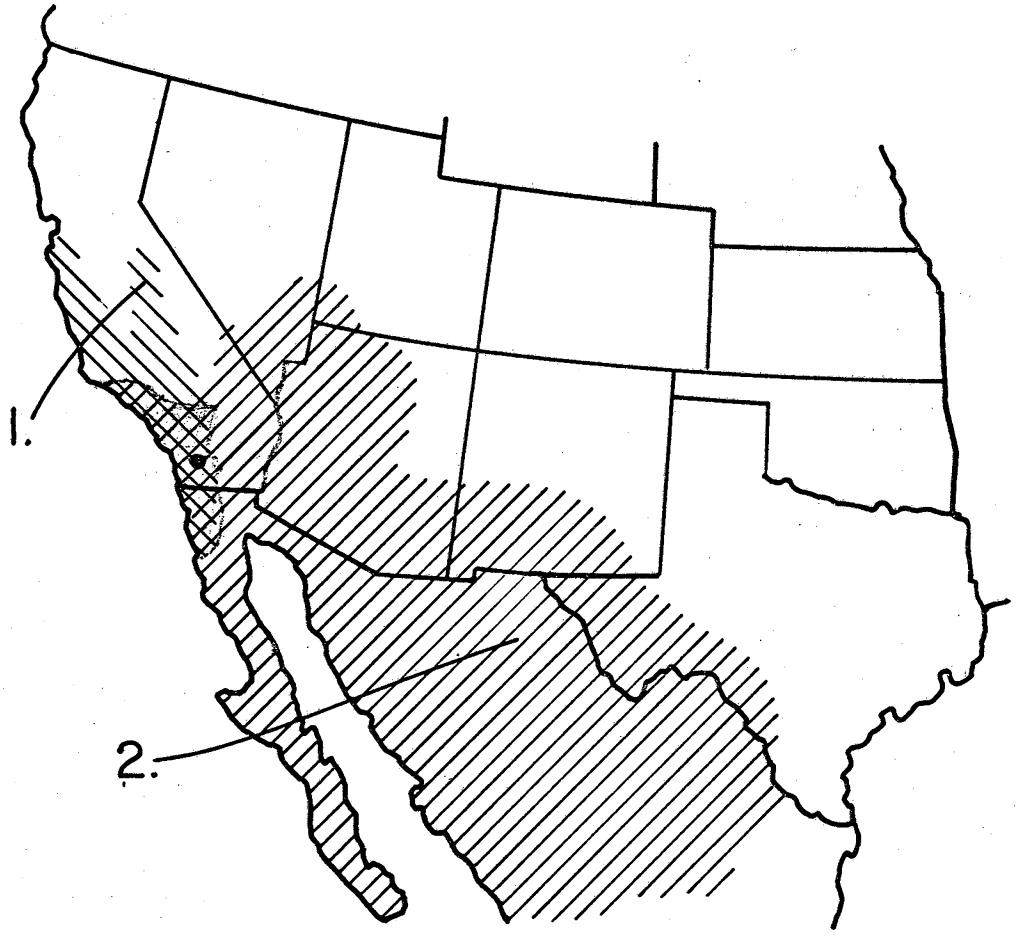
P .1 = +
P .05= *
P .01= **
P .001 =***
P .0001=****

TABLE 5 Multivariant Analysis (General Linear Hypothesis) Listing F-Value and Levels of Significance for Homospecific Choice, Independent Variables, "Initial Choice", and the Interaction of Initial Choice per Species for each 6 Hour Period.

(N = 79)	Six Hour Time Periods									
	1	2	3	4	5	6	7	8	9	10
Homospecific Choice	7.83**	0.76	0.2	0.55	1.02	0.25	1.33	0.48	1.71	0.42
Species	7.59**	4.95*	2.55	2.50	3.81*	1.85	5.78*	0.14	0.00	0.00
Location	4.50***	0.99	0.45	0.39	0.74	0.58	0.81	0.75	1.19	0.82
Season	0.06	0.46	0.25	0.04	0.54	0.32	0.94	0.17	0.00	1.68
Sex	0.53	1.48	0.07	0.39	0.54	0.28	0.19	0.06	0.00	0.21
Initial Choice	9.32**	7.96**	5.40*	3:75*	4.56*	0.66	1.64	1.54	6.27**	3.14*
Species Initial Choice Interaction	0.92	0.57	0.46	0.97	1.03	0.00	0.00	0.18	2.54	0.18
	Night				Night				Night	

P .0001 = ****
P .001 = ***
P . 01 = **
P .05 = *
P .1 = -

Figure 1. Range overlap map for Peromyscus eremicus and Peromyscus californicus. Dot in shaded area indicates approximate location of Loma Linda University and trapping locations.



1. *P. californicus* 2. *P. eremicus*

Figure 2. Schematic drawing of experimental appartus. Compartments A and E housed the stimuli providing animals. Compartments B, C, and D are designated the choice chambers and housed the experimental mice.

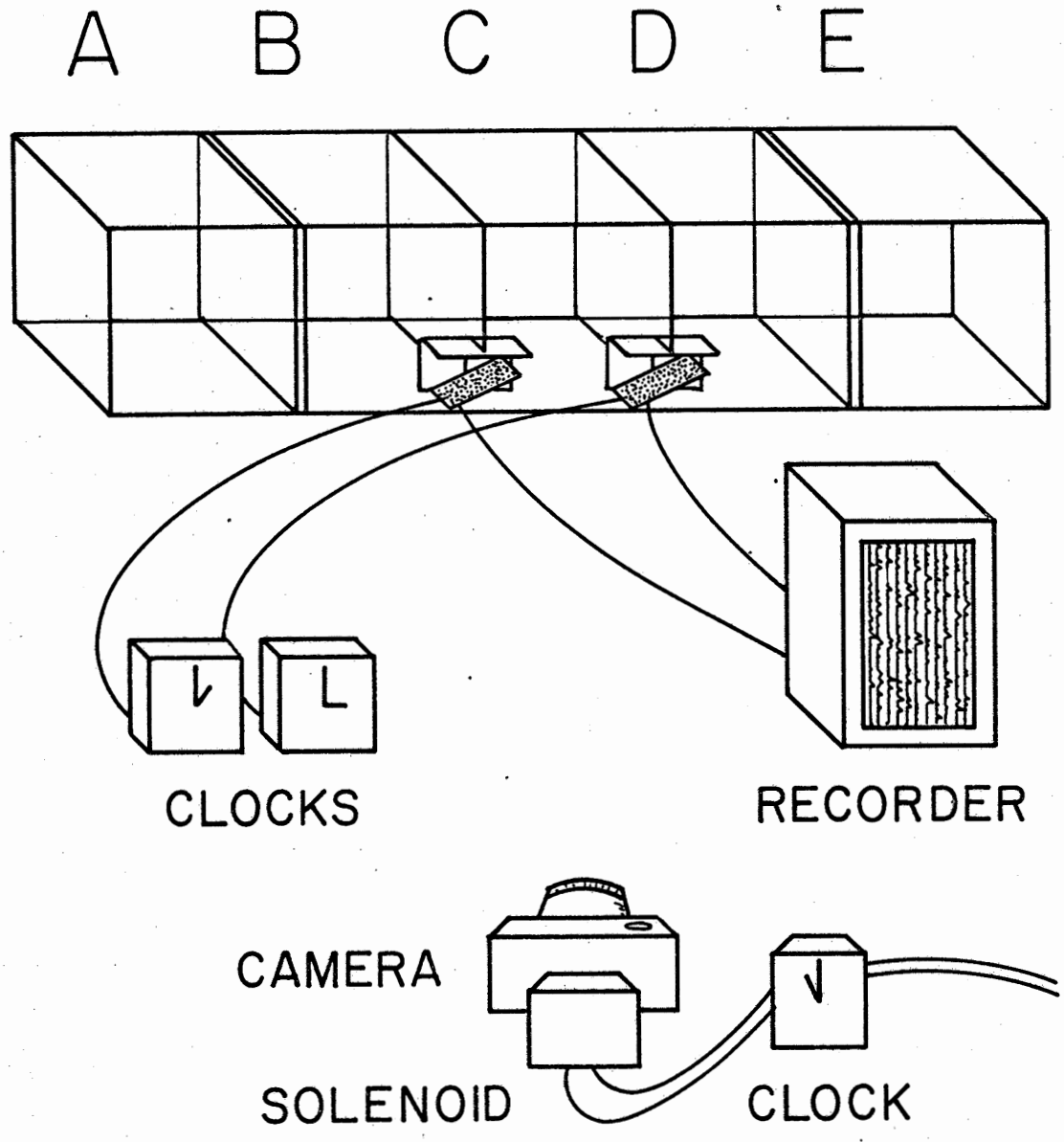

A
B
C
D
E
CLOCKS
RECORDER
CAMERA
SOLENOID
CLOCK

Figure 3. Comparison of mean time in minutes spent in homospecific and heterospecific chambers for pooled data of treatment I (wild caught Peroymscus) (N=131); Bars indicate one standard error above and below the mean (T=4.999; P=.0001; paird-t test).

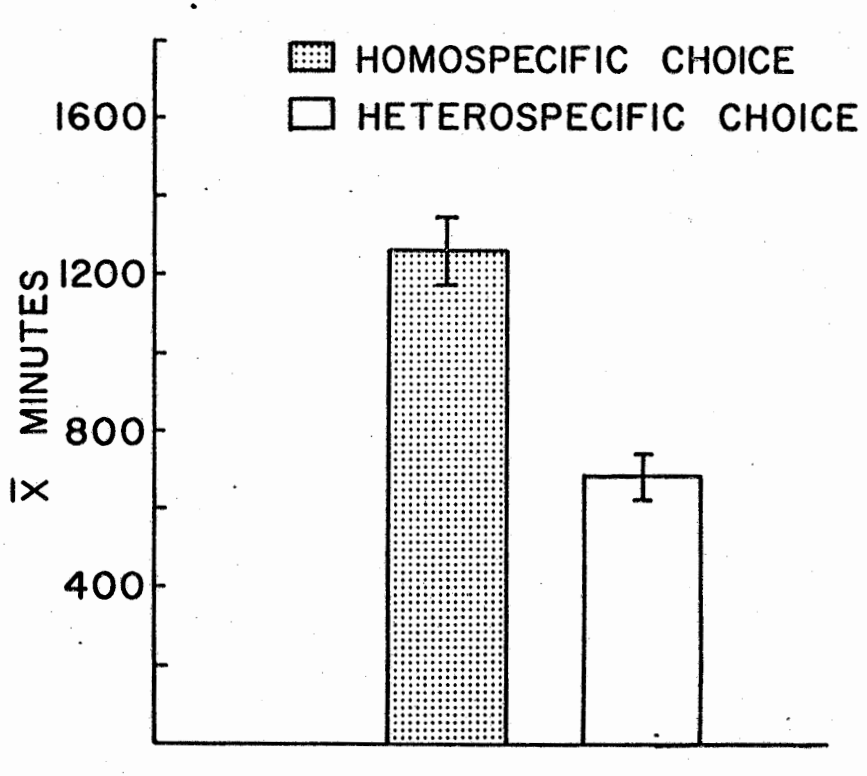

HOMOSPECIFIC CHOICE
HETEROSPECIFIC CHOICE
1600
1200
800
400
$\bar{X}$ MINUTES

Figure 4. Mean number of minutes spent in chambers adjacent to homospecific and heterospecific stimulus mice for the entire testing period (60 hours), comparing subgroups of treatment I (wild caught Peromyscus). The subgroups represent the independent variables: sympatry, allopatry, sex, ans species (P. californicus and P eremicus).

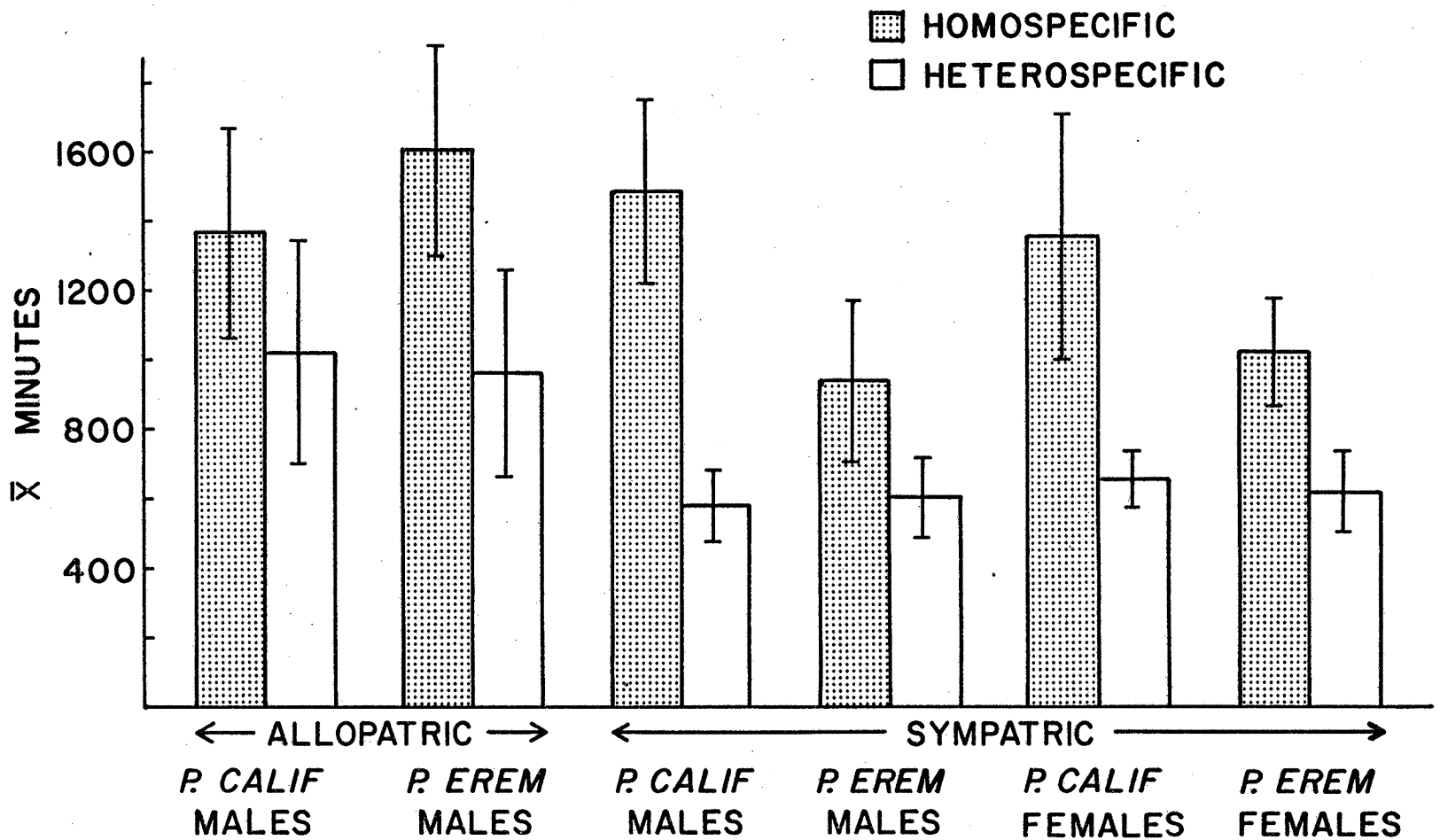

HOMOSPECIFIC
HETEROSPECIFIC
1600
1200
800
400
X̄ MINUTES
← ALLOPATRIC →
← SYMPATRIC →
P. CALIF MALES
P. EREM MALES
P. CALIF MALES
P. EREM MALES
P. CALIF FEMALES
P. EREM FEMALES

Figure 5. Mean number of minutes spent in choice chambers, comparing allopatric and sympatric; males and females; and P. californicus and P. eremicus. Shaded area indicates heterospecific choice, and clear area plus shaded area indicates homospecific choice. (N=131, Bar= one standard error). Comparison 1,2; 3,4; P=(NS). Comparison 5,6; (P=.02).

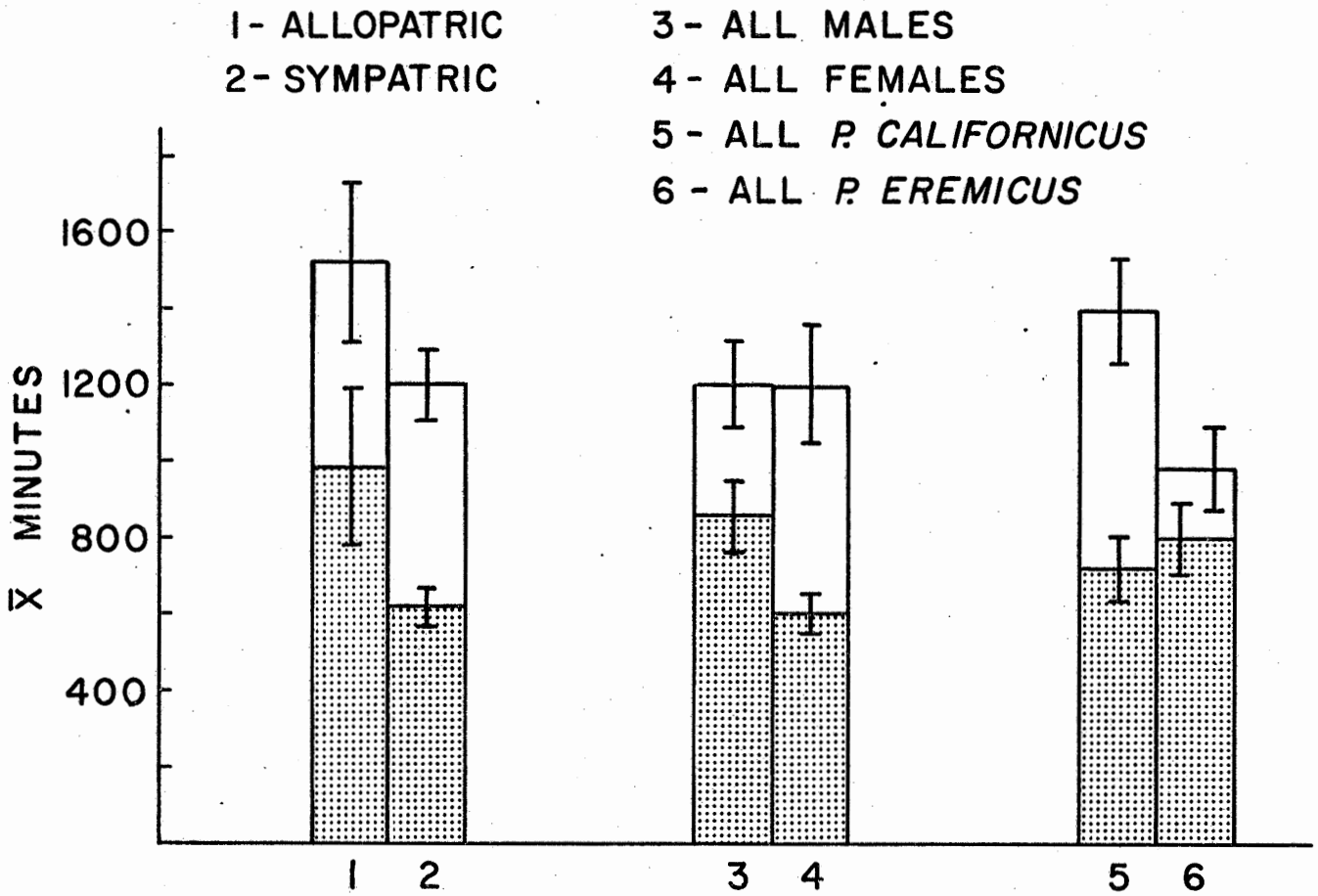

1 - ALLOPATRIC
2 - SYMPATRIC
3 - ALL MALES
4 - ALL FEMALES
5 - ALL P. CALIFORNICUS
6 - ALL P. EREMICUS
1600
1200
800
400
X̄ MINUTES
1
2
3
4
5
6

Figure 6. Comparison of treatment I (wild caught mice) sympatric males and females from both species and their homospecific choice during each ten consecutive six-hour period. Time intervals start with the mean percent choice for the first ninety minutes, followed by mean percent homospecific choice for subsequent six-hour periods (N=79).

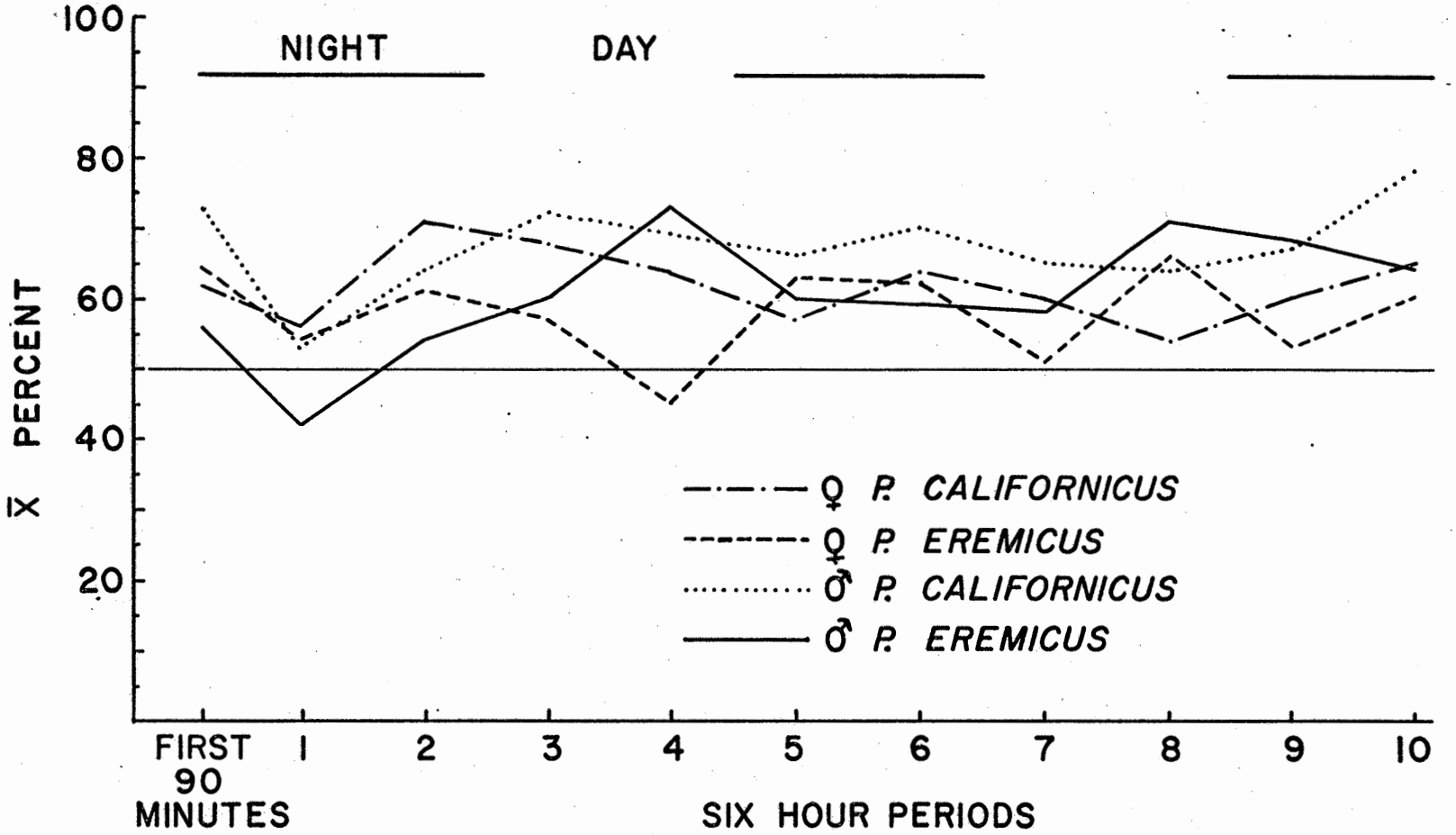

100
80
60
40
20
$\bar{X}$ PERCENT
NIGHT
DAY
♀ P. CALIFORNICUS
♀ P. EREMICUS
♂ P. CALIFORNICUS
♂ P. EREMICUS
FIRST 90 MINUTES
1
2
3
4
5
6
7
8
9
10
SIX HOUR PERIODS

Figure 7. Comparison of mean per cent time in homospecific choice between subgroups of treatment I (wild caught mice) and treatment II (cross-fostered and lab reared controls). Bars indicate one stardard error above and below the mean. Allopatric N=52; sympatric N=79; cross-fostered N=22; and lab reared controls N=22.

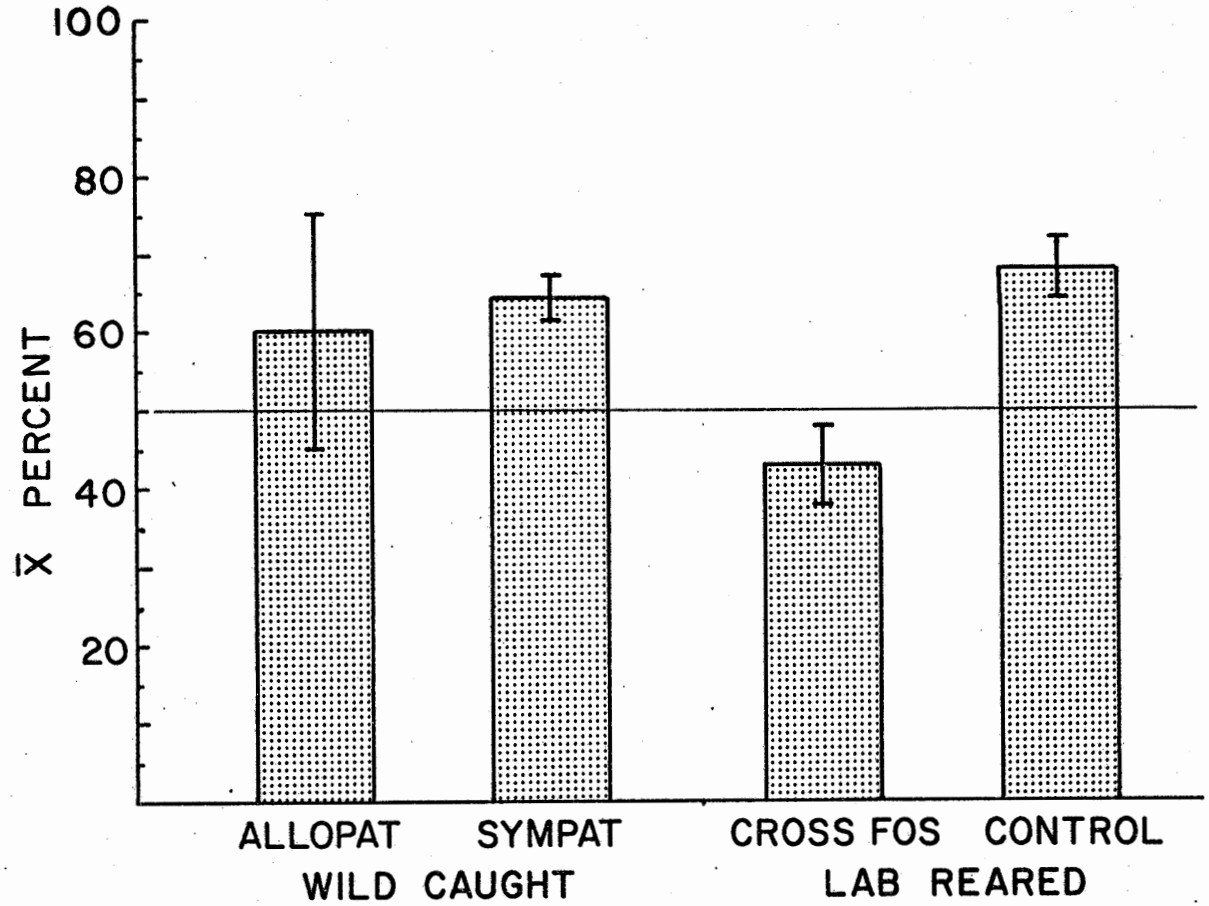
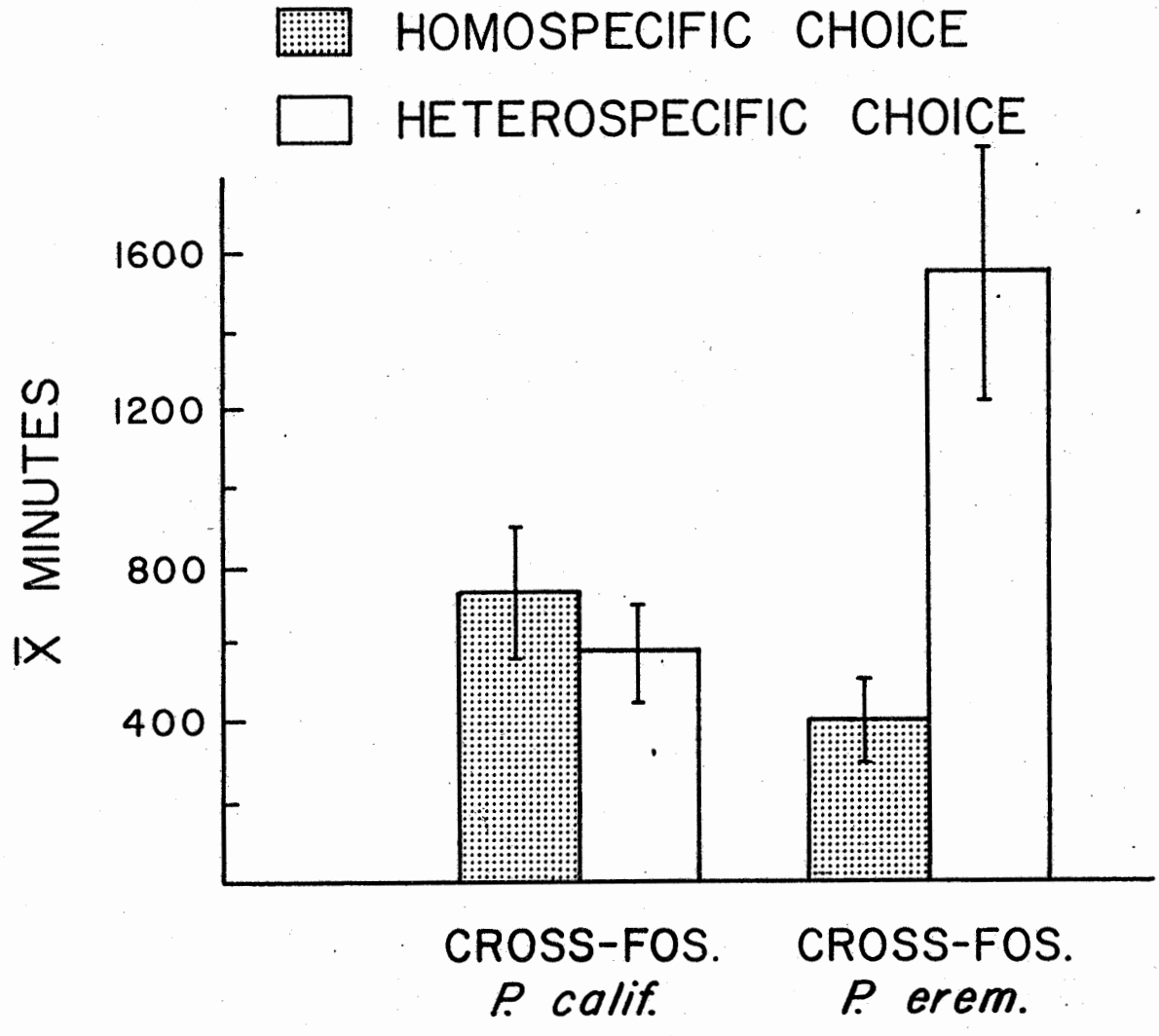

100
80
60
40
20
$\bar{X}$ PERCENT
ALLOPAT
SYMPAT
CROSS FOS
CONTROL
WILD CAUGHT
LAB REARED

Figure 8. Comparison of mean per cent time in homospecific and heterospecific choice for cross-fostered Peromyscus californicus and Peromyscus eremicus. N=22; bars indicate one standard error above and below the mean.


HOMOSPECIFIC CHOICE
HETEROSPECIFIC CHOICE
$\bar{X}$ MINUTES
1600
1200
800
400
CROSS-FOS.
P. calif.
CROSS-FOS.
P. erem.

LITERATURE CITED

Bateson, P.P.G. 1966. The characteristics and context of imprinting. Biol. Rev., 41(2):177-220.

Beach, F.A. 1942. Analysis of the stimuli adequate to elicit mating behaviour in the sexually inexperienced male rat. J. Comp. Psych., 33:163-207.

Beach, F.A., and R.W. Gilmore. 1949. Response of male dogs to urine from females in heat. J. Mammal., 30 (4):391-392.

Blair, W.F. 1953. Experimental evidence of species discrimination in the sympatric species, Peromyscus truei and P. nasutus. Amer. Natur., 87(833):103-105.

Blair, W.F., and W.E. Howard. 1944. Experimental evidence of sexual isolation between three forms of mice of the cenospecies Peromyscus maniculatus. Contr. Lab. Vert. Biol., 26:1-19.

Bowers, J.M., and B.K. Alexander. 1967. Mice: individual recognition by olfactory cues. Science, 158(3805): 1208-1210

Brain, C.L., and G.A. Griffin. 1970. The influences of the sex of littermates on body weight and behaviour in rat pups. Anim. Behav., 18(3):512-516.

Brand, L.R., and R.E. Ryckman. 1969. Biosystematics of Peromyscus eremicus, P. guardia, and P. interparietalis. J. Mammal., 50(3):501-513.

Carr, W.J., and W.F. Caul. 1962. The effect of castration in rat upon discrimination of sex odours. Anim. Behav., 10(1):20-27.

Dagg, A.I. and D.E. Windsor. 1971. Olfactory discrimination limits in gerbils. Can. J. Zool., 49 (3):283-285.

Denenberg, V.H., D.R. Ottinger, and M.W. Stephens. 1962. Effects of maternal factors upon growth and behavior of the rat. Child Devel., 33(1):65-71.

Denenberg, V.H., L.J. Grota, and M.X. Zarrow. 1963. Maternal behaviour in the rat: analysis of cross-fostering. J. Repro. Fert., 5(2):133-141.

Denenberg, V.H., G.A.Hudgens, and M.X. Zarrow. 1964. Mice reared with rats: modification of behavior by early experience with another species. Science, 143(3604):380-381.

Denenberg, V.H., K.M. Rosenberg, R. Paschke, J.L. Hess and M.X. Zarrow. 1968. Plasma corticosterone levels as a function of cross-species fostering and species differences. Endocrinology, 83(4):900-902.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. Amer. Natur., 74(753): 312-321.

Dobzhansky, T. 1951. Genetics and the origin of species. 3rd ed. Columbia Univ. Press, New York, 364 p.

Doty, R.L. 1972. Odor preferences of female Peromyscus maniculatus bairdi for male mouse odors of P. m. bairdi and P. leucopus noveboracensis as a function of estrous state. J. Comp. Physiol. Psych., 81 (2): 191-197.

Doty, R.L. 1973. Reactions of deer mice (Peromyscus maniculatus) and white-footed mice (Peromyscus leucopus) to homospecific and heterospecific urine odors. J. Comp. Physiol. Psych., 84(2):296-303.

Eisenberg, J.F. 1962. Studies on the behavior of Peromyscus maniculatus gambelii and Peromyscus californicus parasiticus. Behaviour 19(2):177-207.

Eisenberg, J.F. 1963. The intraspecific social behavior of some cricetine rodents of the genus Peromyscus. Amer. Midl. Natur., 69(1):240-246.

Fabricius, E. 1962. Some aspects of imprinting in birds. Symp Zool Soc. (London), 8:139-148.

Fenichel, O. 1945. The psychoanalytic theory of neurosis. W.W. Norton and Co., New York, 703 p.

Freud, S. 1949. The infantile genital organization of the libido, p. 244-249. In E. Jones (ed.) The collected papers of Sigmund Freud, vol. 2. Hogarth Press, London, 391 p.

Godfrey, J. 1958. The origin of sexual isolation between bank voles. Royal Physical Soc. (Edinburgh), Prod., 27(1):47-55.

Grota, L.J. 1973. Effects of litter size, age of young, and parity on foster mother behaviour in Rattus norvegicus. Anim. Behav., 21(1):78-82.

Hall, R.E., and K.R. Kelson. 1959. The mammals of North America, vol.2. The Ronald Press Co., New York, 1083 p.

Harris M.P. 1970. Abnormal migration and hybridization of Larus argentatus and L. fuscus after interspecies fostering experiments. Ibis, 112(4):488-498.

Harris, V.T. 1954. Experimental evidence of reproductive isolation between two subspecies of Peromyscus maniculatus. Contr. Lab. Vert. Biol., 70: 1-13.

Heinroth, O. 1911. Beiträge zur Biologie, namentlich Ethologie und Psychologie, der Anatiden. Verh. 5, int. Orn. Kongr. p. 589-702.

Hinde, R.A. 1962. Some aspects of the imprinting problem. Symp. Zool. Soc. (London), 8:129-138.

Hudgens, G.A., V.H. Denenberg, and M.X. Zarrow. 1967. Mice reared with rats: relations between mothers' activity level and offspring's behavior. J. Comp. Physiol. Psych., 63(2):304-308.

Hudgens, G.A., V.H. Denenberg, and M.X. Zarrow. 1968. Mice reared with rats: effects of preweaning and postweaning social interaction upon adult behaviour. Behaviour, 30(1):259-274.

Ingles, L.G. 1965. Mammals of the pacific states. Stanford Univ. Press, Stanford, 506 p.

King, J.A. (ed.) 1968. Biology of Peromyscus (Rodentia). Sp. Pub. No. 2. The Amer. Soc. Mammal, 593 p.

Knight, G.R., A. Robertson and C.H. Waddington. 1956. Selection for sexual isolation within a species. Evolution, 10(1):14-22.

Lorenz, K. 1937. The companion in the bird's world. Auk, 54(3): 245-273.

Marr, J.N., and L.E. Gardner, Jr. 1965. Early olfactory experience and later social behavior in the rat: preference, sexual responsiveness, and care of young. J. Genet. Psychol. 107(1):167-174.

Mayr, E. 1963. Animal species and evolution. Belknap Press of Harvard Univ. Press, Mass., 797 p.

McCabe, T.T., and B.D. Blanchard. 1950. Three species of Peromyscus. Rood Associates, Santa Barbara, Calif., v + 136 p.

McClintock, M.K. 1971. Menstral synchrony and supression. Nature, 229(5282):244-245.

Moltz, H. 1960. Imprinting: empirical basis and theoretical significance. Psych. Bull., 57(4):291-314.

Moore, R.E. 1965. Olfactory discrimination as an isolating mechanism between Peromyscus maniculatus and Peromyscus polionotus. Amer Midl. Natur., 73 (1): 85-100.

Osgood, W.H. 1909. Revision of the mice of the American genus Peromyscus. N. Amer. Fauna, no. 28. U.S. Dept. Ag., Bureau of Biol. Survey. Wash. D.C., 288 p.

Parkes, A.S., and H.M. Bruce. 1961. Olfactory Stimuli in in mammalian reproduction. Science, 134(3485):1049-1054.

Quadagno, D.M., and E.M. Banks. 1970. The effects of reciprocal cross-fostering on the behaviour of two species of rodents, Mus musculus and Baiomys taylori ater. Anim. Behav., 18(2):379-390.

Ressler, R.H. 1962. Parental handling in two strains of mice reared by foster parents. Science, 137(3524): 129-130.

Rosenberg, K.M., V.H. Denenberg, and M.X. Zarrow. 1970. Mice (Mus musculus) reared with rat aunts: the role of rat-mouse contact in mediating behavioural and physiological changes in the mouse. Anim. Behav., 18(1):138-143.

Rugh, R. 1968. The mouse: its reproduction and development. Burgess Pub. Co, Minnesota, 430 p.

Scott, J.P. 1962. Critical periods in behavioral development. Science, 138(3544):949-958.

Selander, R.K. 1965. On mating systems and sexual selection. Amer. Natur., 99(906):129-141.

Sibley, C.G. 1957. The evolutionary and taxonomic significance of sexual dimorphism and hybridization in birds. Condor, 59(3):166-191.

Sluckin W. 1965. Imprinting and early learning. Aldine Pub. Co., Chicago, 147 p.

Sluckin, W., and E.A. Salzen. 1961. Imprinting and perceptual learning. Quart. J. Exp. Psych., 13(2): 65-77.

Smith, M.H. 1965. Behavioral discrimination shown by allopatric and sympatric males of Peromyscus eremicus and Peromyscus californicus between females of the same two species. Evolution, 19(3):430-435.

Spalding, D.A. 1873. Instinct: with original observations on young animals. MacMillian's Mag., 27(160):282-293.

Tamsitt, J.R. 1961. Tests for social discrimination between three species of the Peromyscus truei species group of white-footed mice. Evolution, 15(4):555-563.

Tinbergen, N. 1951. The study of instinct. Clarendon Press, Oxford, 228p.

Tinbergen, N. 1953. Social behaviour in animals. John Wiley and Sons, Inc., New York, 150p.

Todd, J.H., J. Atems, and J.E. Bardach, 1967. Chemical communication in social behavior of a fish, the yellow bullhead (Ictalurus natalis). Science 158 (3801):672-673.

Van der Lee, S., and L.M. Boot. 1955. Spontaneous pseudopregnancy in mice. Acta Physiol. Pharmacol. Neer., 4:442-443.

Walter, M.J. 1973. Effects of parental coloration on the mate preference of offspring in the zebra finch, Taeniopygia guttata castanotis Gould. Behaviour, 46(2):154-173.

Whitman, C.O. 1919. Orthogenetic evolution in pigeons, vol. 3. In H. Carr (ed.) The behavior of pigeons, Carnegie Institute, Wash. Pub. no. 257.

Whitten, W.K., and F.S. Bronson. 1970. The role of pheromones in mammalian reproduction, p. 309-325. In J.W. Johnson, D. G. Moulton, and A. Turk, (ed.) Advances in chemoreception, vol. 1. Communication by chemical signals. Appleton-Centruy-Crofts, New York, 405 p.

APPENDIX A

Species Identification:

Peromyscus californicus and *P. eremicus* are easily identified and separated by numerous body measurements (Ingles, 1965). *Peromyscus californicus* (California mouse) is the larger of the two species with an overall average length of 220-226mm, which is compared to the 170-218mm length of *P. eremicus*. Tail characteristics provide additional information for separating these species. *Peromyscus eremicus* tail measurements range between 89-148mm. Tail hairs are very sparse in *P. eremicus* while tails of *P. californicus* are usually hairy. The overall size difference continues to be apparent in the following key characteristics:

	P. californicus	*P. eremicus*
Hind foot	25-29mm	18-22mm
Ear, notch	20-28mm	18-20mm
Skull	29-32.1mm	24.5mm

The coloring of *P. californicus* is described by Ingles (1965) as "yellowish brown or gray mixed with black above, grayish below; tail . . . unicolored or indistinctly bi-colored, with broad brown dorsal stripe above and lighter brown below; . . .". *Peromyscus eremicus* tend to be more grayish on the upper half of the body with more white below extending down to white feet.

Two additional species, Peromyscus maniculatus (deer mouse) and Peromyscus boylii (brush mouse) have been trapped at the main collection area. Peromyscus maniculatus is easily recognized from the other three species by its reduced size (total length 148-200mm) and by its distinctive tail which in this geographic area is less than its body length (60-90mm and pronouncely bi-colored). Precise identification of the brush mouse has caused some concern since some of the external measurements do overlap with those of P. californicus as seen below:

	P. californicus	P. boylii
length	200-226mm	180-238mm
tail	117-148mm	91-123mm
hindfoot	25- 29mm	20- 26mm
ear	20- 28mm	15- 20mm
skull	29- 32.1mm	27.5-28.5mm

Peromyscus boylii are dark brown to brown above and whitish beneath. The following characteristics proved to be especially helpful in making field identifications; whitish feet with the proximal region of the sole being hairy, and a more or less bi-colored tail. Positive distinction between P. californicus and P. boylii is possible through inspection of the teeth (M 1 , teeth are distinct in the Haplomylomys sub-genus).
only a few P. boylii were trapped. It would have been interesting to test the different mate selection ability

between P. californicus and P. boylii since they are morphologically very similar and since they are from two different sub-genera.

APPENDIX B

Estrus is determined by vaginal smear evaluation. Each stage of estrus (Rugh, 1968) presents distinct cellular characteristics. Stages 1 through 5 are described by the following traits:

1. Almost exclusively leuckocytes, from vaginal smear.
2. Pre-estrus-showing both leukocytes and nucleated cells in approximately equal numbers.
3. Early estrus-showing clearly defined epithelial cell, some with distinct nuclei.
4. Estrus-large, squamous-type epithelial cells without nuclei.
5. Post-estrus-showing approximately equal numbers of leukocytes and epithelial cells, but the latter are large, folded, and with translucent nuclei.

We obtained vaginal smears by means of a pipette, the tip of which had been flamed to a smooth, reduced aperture. A few drops of 0.9% sodium chloride solution are drawn into the pipette. The fluid is transferred to a slide and mounted under a coverslip with a trace of methylene blue to add contrast and bring out the nuclei.